Determining the roles of the *BTB* genes At2g04740, At4g08455, At1g04390, and At2g30600 in *Arabidopsis thaliana* growth and development.

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Introduction

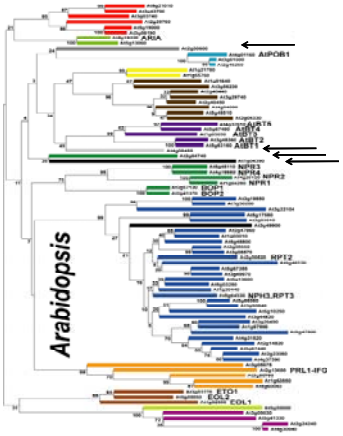
In plants as well as all living organisms the selective degradation of cellular proteins is important for growth and development. This degradation occurs when a cell no longer has a need for an individual protein, either because some change occurs in the environment or in response to developmental cues. Selective protein degradation occurs by activity of the ubiquitin (Ub)/26S proteasome system. In this pathway, proteins to be degraded are tagged with multiple Ubs through the action of three specific enzymes (E1, E2, and E3). The E3 Ub-ligase is the final enzyme in this process as it binds to the target and catalyzes the attachment of the Ub tag to the protein. This tag is then recognized by the 26S proteasome and the protein is degraded (Smalle, 2004).

The Cullin (CUL)-based Ub-ligases are one superfamily of E3s in both plants and animals. In these complexes, the BTB (Bric-a-Brac, Tramtrack, and Broad Complex) domain-containing proteins act as the target adapters, selecting for the proteins to be ubiquitinated (and subsequently degraded) by directly binding to them (Gingerich et al., 2005)(Figure 1). There are a total of 80 BTB proteins encoded in the genome of *Arabidopsis* (Gingerich et al. 2005). In most cases, there are multiple genes in the *BTB* superfamily which encode similar types of BTB proteins, however, there are a few genes in the superfamily that encode for BTB proteins that are unique. In *Arabidopsis*, these genes are At2g04740, At4g08455, At1g04390, and At2g30600. We hypothesize that disruption of just one of these single genes should produce an alteration of plant form or function that we should be able to detect, revealing the function of the gene.

In this project we attempted to identify mutant *Arabidopsis* plants with T-DNA insertions in these four unique genes. We obtained plant lines with putative T-DNA insertions in these genes from the SALK institute in La Jolla, California and screened these using PCR and DNA gel electrophoresis-based genotyping to find individuals with disrupted copies of the genes. We have been observing these mutants under standard growth conditions to determine whether they have any aberrant growth characteristics.

Figure 1. BTB/CUL3 E3 Ubiquitin-Ligase Complex Structure

The *BTB* genes At2g04740, At4g08455, At1g04390, and At2g30600 are unique



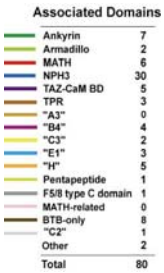


Figure 2. Phylogenic tree of the complete BTB protein superfamily of *Arabidopsis*. *BTB* genes analyzed in this project are indicated by arrows.

T-DNA mutant identification strategy

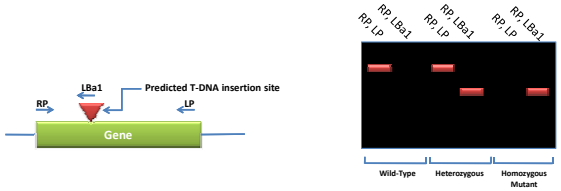


Figure 3. Strategy for identifying individuals with T-DNA insertions in a gene of interest. A population of *Arabidopsis* seedlings that are segregating for the T-DNA insertion is plated on a petri dish with growth medium. The seedlings are allowed to germinate and grow for 7-14 days. Total genomic DNA is isolated from each individual and PCR reactions are performed with RP, LP and RP, LBa1 primer combinations. The PCR products are then electrophoresed in agarose gels for visualization. The diagram above shows what we would expect to see on the gel, depending on whether the individuals are wild-type, heterozygous for the T-DNA insertion, or homozygous for the insertion. Only individuals which are homozygous will have both copies of the gene disrupted.

Results from At1g04390 and At2g04740 genotyping

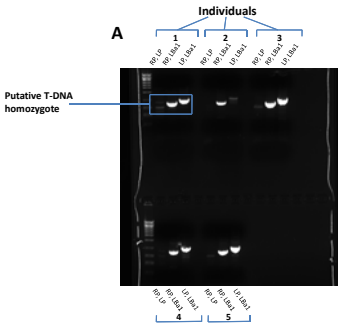


Figure 4. *A*, PCR analysis of DNA isolated from seedlings homozygous for the T-DNA insertion in At1g04390. All 5 samples appear to be homozygous for the T-DNA insertion. *B*, A diagram indicating the putative position of the T-DNA insertion in the At1g04390 gene. Boxes indicate exons and lines indicate introns, respectively. The position of the T-DNA is indicated by a triangle.

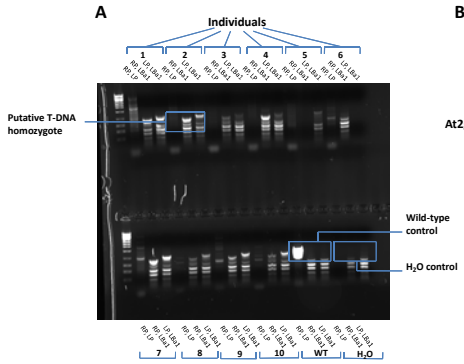


Figure 5. *A*, PCR analysis of DNA isolated from seedlings homozygous for the T-DNA insertion in At2g04740. Wild-type DNA and H_2O are included as controls. *B*, A diagram indicating the positions of the T-DNA insertion in the At2g04740 gene. Boxes indicate exons and lines indicate introns, respectively. The position of the T-DNA is indicated by a triangle.

Conclusions

- We have identified individuals with the At2g04740 and At1g04390 genes disrupted.
- We also identified an insertion in the At2g30600 gene but it appears to be located outside of that gene's coding sequence (and thus may not disrupt gene function).
- Thus far both the At2g04740 and At1g04390 mutant plants appear normal under standard growth conditions.

Selected References

Gingerich, D.J., Gagne, J.M., Salter, D.W., Hellmann, H., Estelle, M., Ma, L., and Vierstra, R.D. (2005). Cullins 3a and 3b assemble with members of the broad complex/tramtrack/bric-a-brac (BTB) protein family to form essential ubiquitin-protein ligases (E3s) in *Arabidopsis*. *J. Biol. Chem.* 280, 18810-18821

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